ON THE OCCURRENCE OF TRYPANOSOMES IN THE BLOOD OF RANA CLAMATA

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WITH ONE PLATE

Some time ago, while investigating the subject of haemosporidia in the blood of frogs, I accidentally came across a spring frog (*Rana clamata*) which was not only abundantly infected with haemosporidia, but was also infected with another form of parasite which upon further investigation was found to belong to the class Monanida, to the family Trypanosomidae, and to the genus *Trypanosoma*. The trypanosomes found in this frog's blood differed so radically from any with which I was acquainted that it was thought a short account of the various forms of organisms observed might be of interest.

Although much has been written upon the morphology of the trypanosomes of warm-blooded animals, very little on the other hand has been said concerning the trypanosomes of cold-blooded animals, and the one receiving the greatest amount of attention at the hands of scientists is the *Trypanosoma rotatorium,* found in the blood of the European edible frog, *Rana esculenta,* and to some extent also in the blood of *Rana temporaria* and *Hyla arborea.*

This trypanosome is described by Stiles (Emergency Report on Surra; Washington, 1902, p. 39) as follows:

"Trypanosoma.—40–80 microns long, by 5–10 microns broad. Flagellum 10–12 microns. Body compressed, semilunate, twisted. The convex border membranous and undulating; the posterior extremity of the body portion pointed, curved inward; the opposite one produced into a long tag or tail-like appendage which almost equals in length the remainder of the body; surface of the body coarsely striate longitudinally; endoplasm or parenchyma slightly granular, endoplast ovate, central."

The trypanosomes observed in the blood of *Rana clamata* were of various shapes, depending upon which stage of their life cycle they had reached, but among these, two forms seemed to predominate, which, briefly described, are as follows:

The smaller of these (pl. 1, fig. 1), which I name *Trypanosoma clamatae,* in reference to the frog in which is was found, is long, slender, and pointed at both ends, and looks very much like an eel while gliding through the blood plasma. It propels itself with a peculiar jerky, wriggling motion, and with sufficient force to easily push aside any blood corpuscles which may happen to be in its path. The anterior end of the parasite bears a long flagellum which is moved so rapidly that it is almost impossible to see it while the parasite is in active motion. The posterior end is extended into a sharp point. The dorsal side of the body is surmounted by an undulating membrane which extends from a point very close to the centrosome to the anterior end of the body. A large oblong nucleus is found on the ventral side of the body, not very far from the anterior end. A large centrosome may be seen at a point almost one-third the way from the posterior end, and occupying nearly the full width of the body.

The flagellum starts at a point very close to the centrosome, follows the outer edge of the undulating membrane till the anterior end of the body is reached, and then becomes free. The body cytoplasm is only slightly granular, and stains easily with polychrom methylen blue and eosin, the Wright stain giving the best results. The body length is about 21 microns, and the diameter 2.5–2.8 microns. The flagellum is 12–13 microns long, thus making the total length of the body and flagellum 33.7–41.7 microns. This organism is the full-grown, or mature trypanosome.

The larger of the two parasites (pl. 1, figs. 4 and 5), which I have already referred to, when observed in the fresh unstained blood, appears of a light greenish color; the body is as a rule semilunate when in a quiescent state, but when in active motion it may assume a great variety of grotesque shapes (pl. 1, fig. 5). The posterior end is pointed, and extends into a tail-like appendage which looks almost like a second flagellum. The convex side or back of the parasite is surmounted by a large undulating membrane, which is kept in constant very rapid motion. The anterior end of the body is provided with a long, slender flagellum, which is likewise kept in constant whip-like motion. The flagellum starts at a point near the centrosome, and extends along the outer edge of the undulating membrane to the anterior end of the body, where it becomes free. The ectoplasm is coarsely granular, while the endo-

plasm is finely granular. A large spherical nucleus is usually found at about the center of the body, but nearer the undulating membrane than the opposite side. The body is also provided with a good-sized centrosome which is located close to the convex side of the parasite, about half way between the nucleus and the posterior end of the body. The body length is 27.56–47 microns, and the diameter at its widest part 16.78–28.51 microns; average length 35.74 microns. The length of the anterior flagellum is 5.96–14.79 microns, the average 10.37 microns. The length of the tail-like appendage is 3.07–4.47 microns, the average 3.77 microns. Therefore, the total length of the parasite, including tail and flagellum, is 44.18 microns. The parasite just described is one of the division forms of *Trypanosoma clamatae.*

I am unable to discover that any attempt has so far been made to work out the life history of the trypanosomes of frogs, and personally I have been no more successful than those who have preceded me in this line of work, but by analogy with *Trypanosoma lewisi,* found in the blood of rats, and whose morphology has been pretty clearly worked out, we know that multiplication of the species may occur by either longitudinal or transverse division, or by segmentation.

In the case of *Trypanosoma clamatae,* I have found that multiplication of the species also takes place by both longitudinal and transverse division, but I have so far failed to observe any signs of multiplication by segmentation.

According to Rabinowitsch and Kempner, after a study of many stained and unstained preparations of *Trypanosoma lewisi,* the centrosome and nucleus of these flagellates is an interdependent whole which corresponds to the nucleus of the other flagellates. They assume that in the early stages of development of the parasite the centrosome and nucleus represent a whole which, as the parasite matures, splits up into two parts which pass to either end of the animal. They also consider the nucleus of the trypanosome as being made up of two parts more or less separated; the small spot situated in the posterior end of the parasite they consider to be a nucleolus, while the larger structure, situated in the front end, they call the chromatin heap.

Wasielewski and Senn, on the other hand, consider that the centrosome is in no way connected with the nucleus. According to their ideas, the trypanosome is made up of two parts, the "plasma"

and the "periplast." The "plasma" is the body of the parasite containing the nucleus. The "periplast" is the outer covering of the parasite and includes the centrosome, flagellum, undulating membrane, and the outer coat investing the body of the organism. According to this hypothesis the centrosome is intimately connected with the undulating membrane, and has nothing to do with Rabinowitsch and Kempner's nucleolus, nor the micro-nucleolus of Plimmer and Bradford.

In multiplication by transverse division in the case of *Trypanosoma lewisi* the first thing to be observed is a change in the outlines of the trypanosome. The sharp beak becomes more blunt and the flagellate end becomes rounded; the long, slender body becomes thickened and swollen with a decided increase in size. There is a multiplication of both nuclei and centrosomes which are arranged in a line parallel to the longitudinal axis of the parent trypanosome. The nuclei and centrosomes now divide, and new flagella are formed, each of which is derived from a centrosome and emerges from the parent parasite on the side which bears the undulating membrane; after a while these attain full length, while the old flagellum becomes destroyed and finally disappears. There is also division of the protoplasm of the parent trypanosome along lines at right angles to the longitudinal axis, so that each new segment of protoplasm is supplied with a nucleus, a centrosome, and a flagellum. The daughter cells soon become liberated in the blood and may be recognized by their small size, but they gradually lengthen out into the characteristic mature organism.

Multiplication by longitudinal division bears a certain analogy to that of transverse division, there being the same change in outline and the same multiplication of nuclei and centrosomes, but the arrangement is in a transverse line parallel to the longitudinal axis. The new flagella make their appearance at the anterior end of the parent parasite, are closely arranged about the old flagellum, and correspond in number to the centrosomes.

The foregoing account of the multiplication of *Trypanosoma lewisi* closely resembles what I have observed in the case of *Trypanosoma clamatae,* though I have not been so fortunate as to view the latter parasite in all its phases of division. From what I have seen, however, I feel confident that multiplication occurs by both longitudinal and transverse division substantially as above set forth.

In what is to follow an attempt will be made to illustrate two

phases in the multiplication of *Trypanosoma clamatae:* (1) The swelling and increase in size of the organism prior to division; (2) The multiplication of the nucleus and centrosome, and the longitudinal and transverse division of the body cytoplasm.

In pl. 1, fig. 2, we have a parasite preparing to divide. When compared with the mature parasite in figure 1, it will be seen that the former has considerably increased in size, both longitudinally and transversely, but as yet it shows no signs of division. In figure 3, we have a trypanosome in a little more advanced stage of multiplication as may be shown by the increased longitudinal and transverse size of the parasite. The nucleus also appears to have divided. In pl. 1, figs. 4 and 5, the two parasites have become so greatly enlarged that it may be fair to assume that they are just on the eve of division, though no actual signs of the multiplication of nuclei and centrosomes, or division of the body protoplasm, are visible. The one in fig. 5 is of particular interest; it represents a parasite of peculiar shape preparing to undergo division; the body is greatly swollen and the centrosome is located nearly in the center of it, while the nucleus is either absent or invisible. In pl. 1, fig. 6, we have a trypanosome in a still more advanced state of division; the body is greatly swollen and enlarged; the nucleus is also greatly enlarged, and evidently preparing to divide; the centrosome, however, shows no signs of multiplication, while the body cytoplasm shows distinct evidence of longitudinal cleavage. In pl. 1, fig. 7, the parasite shown is somewhat similar to that of fig. 8, but in this case the centrosome is clearly seen to be double while the nucleus is very indistinct; the body cytoplasm also shows signs of longitudinal cleavage. In pl. 1, fig. 8, we have a rather peculiar form of division and one which is somewhat difficult to understand. The body of the parasite, as in the preceding illustration, is greatly swollen; the centrosome is intact, but the nucleus seems to have divided. The body cytoplasm clearly shows transverse cleavage, one segment of which, carrying the undulating membrane and flagellum, is probably just about to be split off from the parent trypanosome to form a daughter parasite.

In the foregoing I have tried to show how multiplication in *Trypanosoma clamatae* probably takes place, but, owing to lack of material have been unable to follow the various stages of division to completion. It is to be hoped, however, that further investigation will throw more light upon the subject.

EXPLANATION OF PLATE

Plate I

Photomicrographs illustrating the characters of *Trypanosoma clamatae* nov. sp. Magnified 667 diameters. For explanation see the text.

PLATE I